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Synthesis of new triazole fused imidazo[2,1-*b*]thiazole hybrids with emphasis on *Staphylococcus aureus* virulence factors

Mohd Adil Shareef[a,b], Kanugala Sirisha[b,c], Ibrahim Bin Sayeed[b,c], Irfan Khan[b,c], Thipparapu Ganapathi[d], Syed Akbar[a], C. Ganesh Kumar[b,c], Ahmed Kamal[e,*], Bathini Nagendra Babu[a,b,*]

[a] *Centre for Fluoro-Agrochemicals, CSIR-Indian Institute of Chemical Technology, Tarnaka, 500007 Hyderabad, India*
[b] *Academy of Scientific and Innovative Research, Ghaziabad 201002, India*
[c] *Organic Synthesis and Process Chemistry Division, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500007, India*
[d] *Stem Cell Research Division, National Institute of Nutrition (NIN), Indian Council of Medical Research (ICMR), Hyderabad 500007, Telangana, India*
[e] *School of Pharmaceutical Education and Research (SPER), Jamia Hamdard, New Delhi 110062, India*

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ABSTRACT

A series of new triazole fused imidazo[2,1-*b*]thiazole hybrids (**9a**–**u**) were designed, synthesized and evaluated as antimicrobial agents. Compounds **9c**, **9d**, **9e**, **9j** and **9l** showed promising broad spectrum antimicrobial activity. Further, compound **9c** exhibited significant anti-biofilm activity with single and mixed biofilm disruption demonstrated by Field Emission Scanning Electron Microscope (FE-SEM). Furthermore, molecular docking studies revealed that they interact with the virulence factor, *Staphylococcus aureus* dehydrosqualene synthase (CrtM) (PDB ID: 2ZCS). Overall, the findings suggest compound **9c** as potential lead for further development of novel antibacterial and anti-biofilm agents.

The development of new antibacterial compounds to combat human bacterial infections has never been more alarming and challenging, mainly because of the explicit use of antibiotics and the appearance of bacterial resistance pattern towards a range of antimicrobial agents.[1] The evolution of drug-resistant strains *viz*., methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE) has unfortunately augmented nosocomial and community acquired infections and the inherent mortality rates, which poses an imperative threat to human health.[2] Only four new classes of antibiotics have been approved by FDA over the past 17 years, whilst the majority of current drugs have the same well-understood target.[3,4] In view of these considerations, there is a pressing demand to develop new arsenal of chemotherapeutic agents, and to investigate newer targets or mechanisms to explore the antimicrobial activity.[5]

In this context, efforts were made to explore the dehydrosqualene synthase enzyme which plays a pivotal role in the biosynthesis of golden carotenoid pigment, staphyloxanthin produced by *Staphylococcus aureus*.[6] It is evident that this pigment acts as a virulence factor. It also acts as an antioxidant by shielding the bacterium to endure within the host cell against oxidative stress generated because of host immune defence by neutrophils and reactive oxygen species (ROS).[7] Similarly, persistent biofilms are a key virulence factor, which are recognized as the principal cause of chronic and frequent bacterial infections ensuing an estimated 17 million fresh biofilm infections and more than five hundred thousand deaths each year.[8] Biofilms turn the bacteria resistant to conventional antibiotics by around 1000-fold.[9] Consequently, there is an urgent need to counter biofilm formation and develop new antibacterial agents that can exert anti-biofilm activities.

Imidazo[2,1-*b*]thiazole scaffold has gained renewed interest in view of their extensive pharmacological activities.[10–13] Levamisole, the well known antihelminthic agent (**I**, Fig. 1) is one of the orally active imidazo[2,1-*b*]thiazole derivatives. Besides their clinically proven antihelmintic and immunomodulating actions, various imidazo[2,1-*b*]thiazole derivatives (**II** and **III**, Fig. 1) have exhibited promising antibacterial, antifungal, and anti-tumour activities which has been the primary reason for their inclusion in the hybrid framework.[14–17] Molecular hybridization of imidazo[2,1-*b*]thiazole scaffold with other pharmacophores for the synthesis of newer chemotherapeutics has signified a promising and ongoing field of research in the last few years.

We have been exploring the 1,2,3-triazole motif in our laboratory

* Corresponding authors at: Centre for Fluoro-Agrochemicals, CSIR-Indian Institute of Chemical Technology, Tarnaka, 500007 Hyderabad, India (B. Nagendra Babu).
E-mail addresses: pvc@jamiahamdard.ac.in (A. Kamal), bathini@iict.res.in (B. Nagendra Babu).

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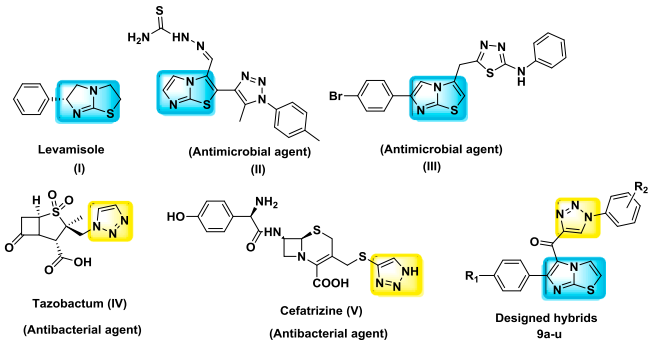
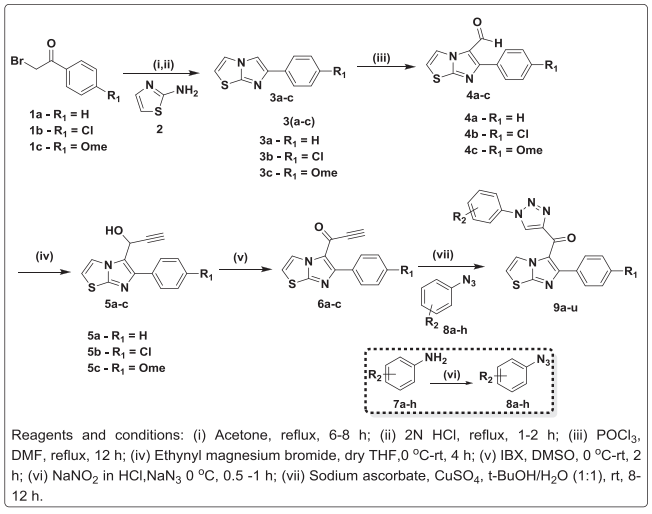


Fig. 1. Structures of compounds containing imidazo[2,1-*b*]thiazole and 1,2,3-triazole motifs.

for the development of newer chemotherapeutic agents.[18] In addition, when pooled with other heterocycles, it contributes in enhancing the biological activity, as it efficiently binds with various biological targets *via* hydrogen bonds, pi-stacking interactions and dipole-dipole interactions.[19] Moreover, 1,2,3-triazole motif can be easily synthesized and is an integral structural motif of various marketed antibacterial agents such as Tazobactam **(IV)** and Cefatrizine **(V)** (Fig. 1). Despite significant investigations on 1,2,3-triazoles, continuous efforts are still being made to integrate them in exploring newer agents with potent broad spectrum antibacterial and anti-biofilm activities.

In view of the above consideration and in furtherance to our ongoing efforts towards the discovery of new potent heterocyclic chemotherapeutic agents, new triazole fused imidazo[2,1-*b*]thiazole hybrids were designed *via* molecular hybridization by incorporating phenyl triazoles at the 5th position of imidazo[2,1-*b*]thiazole scaffold.[20] Further evaluated for their *in vitro* antimicrobial activity against an array of microorganisms and the most promising candidates were further studied for biofilm inhibition potential.

Synthesis of triazole fused imidazo[2,1-*b*]thiazole hybrids (**9a–u**) is represented in Scheme 1. Initially, equimolar quantities of substituted 2-bromoacetophenones (**1a–c**) and 2-aminothiazole (**2**) were reflux for 6–8 h followed by addition of 2 N HCl and continued the reflex for another 1–2 h to obtain imidazo[2,1-*b*]thiazoles (**3a–c**). These obtained intermediates were further subjected to Vilsmeier-Haack reaction conditions to afford the corresponding imidazothiazole aldehydes (**4a–c**) and the resulting aldehydes were then reacted with ethynylmagnesium bromide in dry THF to gain the intermediates (**5a–c**) which upon oxidation by means of 2-iodoxybenzoic acid (IBX) in DMSO provided the corresponding terminal alkyne precursors (**6a–c**). Similarly, substituted



Scheme 1. Synthesis of triazole fused imidazo[2,1-*b*]thiazole hybrids (**9a–u**).

phenyl azides (**8a–g**) were prepared in one pot from the corresponding anilines (**7a–g**) as per the literature protocol.[21] Finally, the title compounds (**9a–u**) were synthesized by reaction of corresponding precursors (**6a–c**) with phenyl azides (**8a–g**) in presence of catalytic amount of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate in tertiary butanol/water mixture as click chemistry protocol[22] in good to excellent yields. All the synthesized hybrids were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS spectral data.

All the synthesized triazole fused imidazo[2,1-*b*]thiazole hybrids (**9a–u**) were screened for their antimicrobial activity[23] and the results are represented in Table 1. Among them, compounds **9c**, **9d**, **9e**, **9j** and **9l** demonstrated promising broad spectrum antibacterial activity against all the bacterial strains with MIC values ranging between 1.9 and 7.8 μg/mL and also displayed moderate antifungal activity with MIC values ranging between 7.8 and 15.6 μg/mL.

Structure–activity relationship (SAR) study revealed that that amongst the synthesized triazole fused imidazo[2,1-*b*]thiazole hybrids, compounds (**9o–u**) with electron donating substituent such as 4-methoxy group at $\mathbf{R_1}$ position exhibited diminished antibacterial activity, while the compounds (**9h–n**) with 4-chloro substitution displayed better antibacterial activity whereas compounds (**9a–g**) without any substitution ($\mathbf{R_1}$) demonstrated promising antibacterial activity. On the contrary, compounds **9c**, **9d**, **9e**, **9j**, **9k** and **9l** with substituents like 4-chloro, 2-bromo 4-fluoro and 4-bromo groups on the phenyl ring of triazole exhibited pronounced antibacterial activity in comparison with the compounds **9a**, **9b**, **9h**, **9i**, **9o** and **9p** bearing substituents such as 3,4,5-trimethoxy and 4-methoxy groups ($\mathbf{R_2}$). Moreover, compounds (**9f**, **9m** and **9t**) with trifluoromethyl group were found to be having moderate antibacterial activity against gram positive bacteria and diminished activity against gram negative bacteria.

Based on the significant antimicrobial activity demonstrated by these hybrids, they were further screened for minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) against various microbial strains[24] and the results are represented in Table 2. Compounds such as **9c**, **9d**, **9e**, **9j** and **9l** demonstrated promising broad spectrum activity against all the tested bacterial strains with MBC values in the range of 3.9–15.6 μg/mL. Minimum fungicidal concentration (MFC) values of these promising compounds were found to be ranging amid 15.6 and 31.2 μg/mL.

Keeping in view of the significance of biofilms, we tested our most active hybrids (compounds **9c**, **9d**, **9e**, **9j** and **9l**) for their biofilm inhibition property.[25] All the tested hybrids were found to possess promising biofilm inhibition property. The results are represented in Table 3. The results revealed that compounds **9c**, **9d**, **9e**, **9j** and **9l** displayed promising biofilm inhibition activity in the range of 9.8–21.1 μg/mL for the tested bacterial biofilms. Fascinatingly, compound **9c** remarkably exhibited significant anti-biofilm activity against the entire tested bacterial biofilms. Biofilm IC_{50} (Half maximal inhibitory concentration) of compound **9c** was found to be 9.8 and 10.3 μg/mL against *S. aureus* MTCC 96 and *E. coli* MTCC 739 strains, respectively, while the IC_{50} value of standard ciprofloxacin was found to be 6.7 and 7.3 μg/mL, respectively.

The effect of compound **9c** on biofilm formation was studied by FE-SEM.[26] The results to this regard are depicted in Fig. 2(a–h). FE-SEM micrographs of the control biofilms of *S. aureus* MTCC 96 (Fig. 2a) with no treatment depicted an intact biofilm with unaltered cell surface and morphology, while *S. aureus* MTCC 96 biofilms treated with compound **9c** at dose of its MBC clearly indicated significant disruption and cell damage (Fig. 2b). Fig. 2c depicts the control group of *E. coli* MTCC 739 with intact cell morphology. Whereas, the **9c** treated biofilms of *E. coli* MTCC 739 showed lysis of cells with a damaged membrane as seen in the Fig. 2d. The mixed biofilms of *S. aureus* MTCC 96 and *E. coli* MTCC 739 with normal morphology and secreted biofilm matrix are depicted in the Fig. 2e and g. The mixed biofilms of *S. aureus* MTCC 96 and *E. coli* MTCC 739 treated with the derivative **9c** with the biofilm matrix disrupted and the individual cells lysed are depicted in the Fig. 2f and h.

Table 1
Antimicrobial activity of the synthesized triazolo fused imidazo[2,1-*b*]thiazole hybrids (**9a–u**).

Test Compds	R_1	R_2	Minimum inhibitory concentration (μg/mL)							
			Gram positive bacteria				Gram negative bacteria			Fungus
			B. s [a]	*S. a* [b]	*M. l* [c]	*S. a* [d]	*E. c* [e]	*P.a* [f]	*K. p* [g]	*C .a* [h]
9a	H	3,4,5-Tome	3.9	7.8	3.9	3.9	> 125	> 125	31.2	15.6
9b	H	4-OCH_3	3.9	7.8	3.9	7.8	> 125	> 125	15.6	15.6
9c	H	4-Cl	3.9	1.9	3.9	1.9	1.9	3.9	3.9	7.8
9d	H	2-Br 4-F	3.9	1.9	3.9	1.9	1.9	3.9	3.9	7.8
9e	H	4- Br	3.9	3.9	7.8	3.9	3.9	3.9	3.9	7.8
9f	H	4-CF_3	3.9	7.8	3.9	3.9	> 125	> 125	> 125	15.6
9g	H	4-F	7.8	> 125	> 125	> 125	> 125	> 125	> 125	15.6
9h	Cl	3,4,5-Tome	3.9	7.8	15.6	7.8	> 125	> 125	> 125	15.6
9i	Cl	4-OCH_3	3.9	7.8	3.9	3.9	> 125	> 125	3.9	7.8
9j	Cl	4-Cl	3.9	7.8	3.9	3.9	3.9	3.9	3.9	7.8
9k	Cl	2-Br 4-F	3.9	7.8	3.9	3.9	> 125	> 125	3.9	7.8
9l	Cl	4- Br	3.9	7.8	3.9	3.9	3.9	3.9	3.9	7.8
9m	Cl	4-CF_3	15.6	> 125	> 125	> 125	> 125	> 125	> 125	15.6
9n	Cl	4-F	3.9	7.8	3.9	3.9	> 125	> 125	7.8	15.6
9o	OCH_3	3,4,5-Tome	15.6	7.8	15.6	15.6	> 125	> 125	> 125	7.8
9p	OCH_3	4-OCH_3	7.8	7.8	15.6	15.6	> 125	> 125	7.8	15.6
9q	OCH_3	4-Cl	3.9	7.8	3.9	3.9	> 125	> 125	> 125	15.6
9r	OCH_3	2-Br 4-F	7.8	7.8	3.9	3.9	> 125	> 125	15.6	7.8
9s	OCH_3	4- Br	7.8	7.8	7.8	3.9	> 125	> 125	15.6	7.8
9t	OCH_3	4-CF_3	15.6	15.6	7.8	15.6	> 125	> 125	> 125	7.8
9u	OCH_3	4-F	3.9	7.8	3.9	3.9	> 125	> 125	> 125	15.6
CPF	–	–	0.9	0.9	0.9	0.9	0.9	0.9	0.9	–[a]
MCZ	–	–	–[a]	–	–	–	–	–	–	3.9

–[a] No activity.
CPF, Ciprofloxacin and **MCZ,** Miconazole as reference drugs (Controls). The tests were done in triplicates and represented as mean values for [a] *Bacillus subtilis* MTCC 121; [b] *Staphylococcus aureus* MLS-16 MTCC 2940; [c] *Micrococcus luteus* MTCC 2470; [d] *Staphylococcus aureus* MTCC 96; [e] *Escherichia coli* MTCC 739; [f] *Pseudomonas aeruginosa* MTCC 2453; [g] *Klebsiella planticola* MTCC 530 and [h] *Candida albicans* MTCC 3017.

Table 2
Minimum bactericidal/fungicidal concentration (μg/mL) of the synthesized triazolo fused imidazo[2,1-*b*]thiazole hybrids (**9a–u**).

Test Compds	Minimum bactericidal/fungicidal concentration (μg/mL)							
	Gram positive bacteria				Gram negative bacteria			Fungus
	B. s [a]	*S. a* [b]	*M. l* [c]	*S. a* [d]	*E. c* [e]	*P. a* [f]	*K. p* [g]	*C .a* [h]
9a	7.8	15.6	7.8	7.8	> 125	> 125	62.5	31.2
9b	7.8	15.6	7.8	15.6	> 125	> 125	31.2	31.2
9c	7.8	3.9	7.8	3.9	3.9	7.8	7.8	15.6
9d	7.8	3.9	7.8	3.9	3.9	7.8	7.8	15.6
9e	7.8	7.8	15.6	7.8	7.8	7.8	7.8	15.6
9f	7.8	15.6	7.8	7.8	> 125	> 125	> 125	31.2
9g	15.6	> 125	> 125	> 125	> 125	> 125	> 125	31.2
9h	7.8	15.6	31.2	15.6	> 125	> 125	> 125	31.2
9i	7.8	15.6	7.8	7.8	> 125	> 125	7.8	15.6
9j	7.8	15.6	7.8	7.8	7.8	7.8	7.8	15.6
9k	7.8	15.6	7.8	7.8	> 125	> 125	7.8	15.6
9l	7.8	15.6	7.8	7.8	7.8	7.8	15.6	15.6
9m	31.2	> 125	> 125	> 125	> 125	> 125	> 125	15.6
9n	7.8	15.6	7.8	7.8	> 125	> 125	15.6	> 125
9o	31.2	15.6	31.2	31.2	> 125	> 125	> 125	15.6
9p	15.6	15.6	31.2	31.2	> 125	> 125	15.6	31.2
9q	7.8	15.6	7.8	7.8	> 125	> 125	> 125	31.2
9r	15.6	15.6	7.8	7.8	> 125	> 125	15.6	15.6
9s	15.6	15.6	15.6	7.8	> 125	> 125	31.2	15.6
9t	31.2	31.2	15.6	31.2	> 125	> 125	> 125	15.6
9u	7.8	15.6	7.8	7.8	> 125	> 125	> 125	31.2
CPF	1.9	1.9	1.9	1.9	1.9	1.9	1.9	– [a]
MCZ	–[a]	–	–	–	–	–	–	3.9

–[a] No activity; CPF, Ciprofloxacin and MCZ, Miconazole as reference drugs (Controls). The tests were done in triplicates and represented as mean values for [a] *Bacillus subtilis* MTCC 121; [b] *Staphylococcus aureus* MLS-16 MTCC 2940; [c] *Micrococcus luteus* MTCC 2470; [d] *Staphylococcus aureus* MTCC 96; [e] *Escherichia coli* MTCC 739; [f] *Pseudomonas aeruginosa* MTCC 2453; [g] *Klebsiella planticola* MTCC 530; and [h] *Candida albicans* MTCC 3017.

Table 3
Biofilm inhibitory concentration (μg/mL) of the synthesized triazolo fused imidazo[2,1-*b*]thiazole hybrids **(9a–u)**.

Compound	Biofilm inhibitory concentration (μg/mL)						
	B. s [a]	*S. a* [b]	*M. l* [c]	*S. a* [d]	*E. c* [e]	*P. a* [f]	*K. p* [g]
9c	11.4 ± 0.22	10.63 ± 0.03	11.23 ± 0.20	**9.8 ± 0.08**	**10.3 ± 0.20**	14.2 ± 0.8	14.6 ± 0.09
9d	13.5 ± 0.09	12.8 ± 0.08	12.3 ± 0.06	13.5 ± 0.15	18.2 ± 0.20	18.2 ± 0.8	17.3 ± 0.08
9e	16.0 ± 0.26	14.4 ± 0.07	13.9 ± 0.09	21.8 ± 0.07	20.2 ± 0.20	20.2 ± 0.07	21.1 ± 0.08
9j	13.2 ± 0.06	11.9 ± 0.08	13.7 ± 0.08	18.4 ± 0.03	14.0 ± 0.24	19.3 ± 0.06	16.8 ± 0.18
9l	13.5 ± 0.08	14.0 ± 0.06	16.0 ± 0.04	21.3 ± 0.06	16.2 ± 0.8	17.5 ± 0.05	20.5 ± 0.06
CPF	6.6 ± 0.06	6.6 ± 0.06	6.8 ± 0.05	6.7 ± 0.05	7.3 ± 0.04	7.4 ± 0.04	8.6 ± 0.04

CPF, Ciprofloxacin**,** as reference drug (Control). The tests were carried out in triplicates and represented as mean values for [a] *Bacillus subtilis* MTCC 121. [b] *Staphylococcus aureus* MLS-16 MTCC 2940. [c] *Micrococcus luteus* MTCC 2470. [d] *Staphylococcus aureus* MTCC 96. [e] *Escherichia coli* MTCC 739. [f] *Pseudomonas aeruginosa* MTCC 2453. [g] *Klebsiella planticola* MTCC 530. Bold signifies promising values.

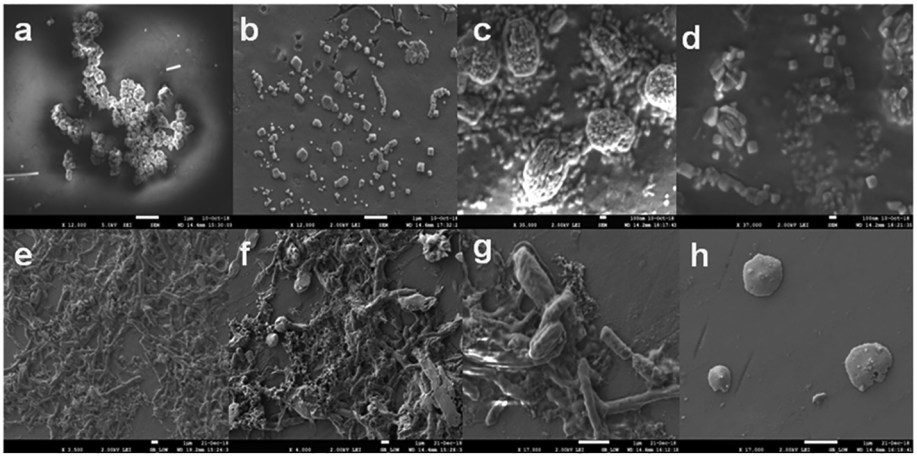


Fig. 2. (a–h) Effect of compound **9c** on the dual species biofilms formed by *S. aureus* MTCC 96 and *E. coli* MTCC 739; a. *S. aureus* biofilm untreated; b. *S. aureus* biofilm treated with **9c** depicting the lysis of *S. aureus* MTCC 96; c. *E. coli* biofilm untreated; d. *E. coli* biofilm treated with **9c** depicting the lysis of *E. coli* MTCC 739; e. Dual species biofilm of *S. aureus* and *E. coli,* untreated (4000×); f. Dual species biofilm of *S. aureus* and *E. coli,* treated with **9c** (4000×); g. Dual species biofilm of *S. aureus* and *E. coli,* untreated (17000×); h. Dual species biofilm of *S. aureus* and *E. coli,* treated with **9c** (17000×).

In the current study, the dual species biofilm inhibition was assessed for the compound **9c** by testing against *Staphylococcus aureus* MTCC 96 and *Escherichia coli* MTCC 739 dual species biofilms.[27] The results to this context are represented in Table 4. The results revealed that mixed biofilm IC_{50} of compound **9c** was found to be 14.3 μg/mL as compared to ciprofloxacin which exhibited an IC_{50} of 11.6 μg/mL.

Furthermore, the most potential compounds (**9c**, **9d**, **9e**, **9j** and **9l**) were evaluated for their *in vitro* cytotoxicity against normal human lung cell line MRC5 (ATCC No. CCL 171) using MTT assay[28] and the results are represented in Table 5. In this assay, ciprofloxacin was used as positive control along with DMSO as negative control. IC_{50} values are represented in μg/mL as mean ± S.D. The results revealed that the cytotoxicity of these compounds was found to be in the range of 24–37 μg/mL while their antibacterial activity (MIC) was found to be in the range of 1.9–7.8 μg/mL. These findings suggests that compound **9c**, **9d**, **9e**, **9j** and **9l** exhibited lower cytotoxicity to normal cell lines compared to the antibacterial activity.

The promising antibacterial results encouraged us to further investigate the possible mode of action. In view of the importance of dehydrosqualene synthase (CrtM) as a virulence factor,[29,30] the molecular docking studies were carried out on this enzyme. The three dimensional prote in crystal structure of the *S. aureus* C(30) carotenoid dehydrosqualene synthase complexed with bisphosphonate BPH-700 (PDB ID: 2ZCS) was obtained from protein data bank.[31] Docking studies were carried out to identify the Protein–Ligand interactions of the most active compound**s** namely **9c**, **9d**, **9e**, **9j** and **9l** using Molegro Virtual Docker.[32]

Investigation of the receptor ligand complex models was carried out based on the parameters such as MolDock score, H–Bond energy, Protein–Ligand interactions and Water–Ligand interactions of the docked compound inside the active site. The results are represented in Table 6 and the Protein–Ligand interactions were represented in Fig. 3. The amino acid residues present in the active binding site of the protein 2ZCS can interact with these compounds which evidently reveal the binding positions of ligands with the protein.

Interestingly, compounds **9c**, **9d**, **9e**, **9j** and **9l** were found to be binding in a similar orientation and with the same binding mode when superimposed as shown in Supplementary Fig. S3. The key ligand interactions (steric) with the amino acid residues of the target include Arg45, Phe22, Gln165, Gly161, Leu164, Leu141, Tyr41, Val137, Ala134, His18 and Asn168. Almost, all the docked poses interact with these residues, which indicate that this active site is the most favorable binding site for the compounds. None of the compounds showed H-

Table 4
Mixed biofilm inhibitory concentration (μg/mL) of compound **9c.**

Test Compd	Dual biofilm inhibitory concentration[ǂ] (μg/mL)
9c	14.3 ± 0.26
Ciprofloxacin	11.6 ± 0.19

ǂ *S. aureus* MTCC 96 + *E. coli* MTCC 739.

Table 5
Cytotoxicity assay of the most active derivatives (**9c**, **9d**, **9e**, **9j** and **9l**) on MRC5 normal cell line.

Compound code	IC_{50} values (μg/mL)
9c	36.1 ± 0.5
9d	48.2 ± 0.9
9e	28.1 ± 0.2
9j	24.2 ± 0.2
9l	37.3 ± 0.4
Ciprofloxacin	53.4 ± 0.3

Table 6
MolDock score, H–Bond energy, Protein–Ligand interactions and Water–Ligand interactions of the most active compounds (**9c**, **9d**, **9e**, **9j** and **9l**) and antimicrobial Agent II with the target (**PDB**: **2ZCS**).

Code	MolDock Score	Protein–Ligand interactions	Hydrogen bonds	Water–Ligand interactions
9c	−141.546	−126.757 [Arg45, Phe22, Gln165, Gly161, Leu164, Leu141, Tyr41]	0	−14.789 [HOH334, HOH353, HOH568]
9d	−138.455	−130.363 [Val137, Leu141, Gly161, Leu164, Ala134, Gln165, Phe22, His18]	0	−8.092 [HOH334, HOH353, HOH568]
9e	−139.608	−129.461 [Leu141, Leu164, Gly161, Phe22 Ala134, Gln165, His18]	0	−10.147 [HOH334, HOH353, HOH568]
9j	−141.545	−127.338 [Arg45, Gln165, Asn168, Ala134, Phe22, Gly161, Leu164, Val137]	0	−14.207 [HOH334, HOH353, HOH568]
9l	−141.81	−132.742 [Leu141, Gly161, Leu164, Ala134, Phe22, Gln165, Asn168]	0	−9.068 [HOH334, HOH353, HOH568]
Agent II	−157.702	−137.126 [Leu164, Leu141, Gly138, Asp48, Val133, Ala134]	−2.952 [Tyr248, Gln165]	−17.624 [HOH334, HOH568]

bond interactions with amino acid residues. However, they exhibited strong H–Bond interactions with co-crystal water molecules (HOH334, HOH353, HOH568) which also play an important role in ligand binding with target.[33,34] Among the docked compounds, compound **9c** and **9j** displayed significant Water–Ligand interactions while the antimicrobial agent II showed strong H–Bond interactions with amino acid residues (Tyr248, Gln165) and co-crystal water molecules (HOH334, HOH568) apart from steric interactions. Overall, molecular docking studies provided valuable molecular insights about the interactions and binding modes of most active compounds with dehydrosqualene synthase enzyme.

In conclusion, a series of new triazolo fused Imidazo[2,1-*b*]thiazole hybrids **(9a–u)** were synthesized and evaluated for their *in vitro* antibacterial, MBC, antifungal, MFC and biofilm inhibition activities. Among them, the hybrids **9c**, **9d**, **9e**, **9j** and **9l** demonstrated promising broad spectrum antibacterial activity against the entire set of tested pathogens with MIC values ranging between 1.9 and 7.8 µg/mL and displayed moderate antifungal activity with MIC values ranging between 7.8 and 15.6 µg/mL. In addition, compounds **9c**, **9d**, **9e**, **9j** and **9l** demonstrated promising broad spectrum activity against all the tested bacterial strains with MBC values ranging between 3.9 and 15.6 µg/mL. Further, biofilm inhibition assay revealed that compounds **9c**, **9d**, **9e**, **9j** and **9l** displayed promising biofilm inhibition in the tested bacterial biofilms, especially the compound **9c** exhibited significant anti-biofilm activity against all the tested bacterial biofilms and its IC_{50} was found to be 9.8 and 10.3 µg/mL against *S. aureus* MTCC 96 and *E. coli* MTCC 739 strains, respectively. FE-SEM micrographs clearly indicated that the compound **9c** caused biofilm disruption and was found to be a promising biofilm inhibitor. Furthermore, mixed biofilm assay revealed that the dual species mixed biofilms were effectively disrupted by compound and the mixed biofilm IC_{50} of compound **9c** was found to be 14.3 µg/mL. Further, MTT assay revealed that the most potential compounds were non toxic to MRC5 normal cell line. Moreover, docking studies predicted the binding modes of the most potential compounds with the target enzyme. Therefore, these findings offer insights to design and develop new antibacterial leads with emphasis on the virulence factors such as biofilms and dehydrosqualene synthase (CrtM) of *S. aureus*.

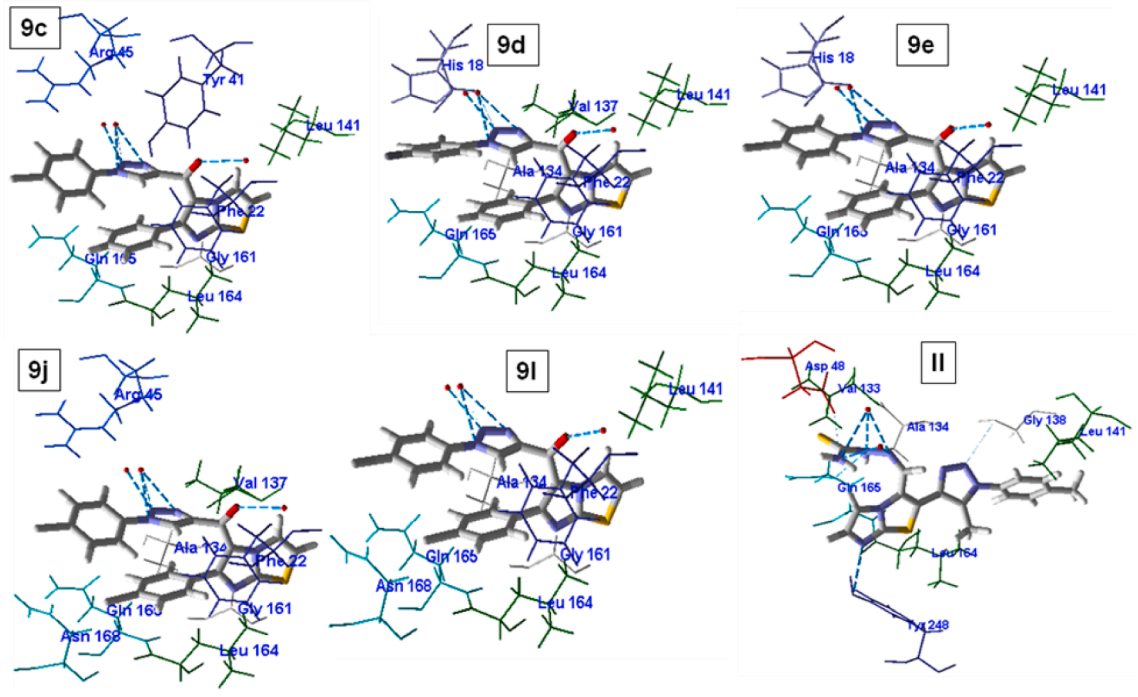


Fig. 3. Docking interactions of compounds **9c**, **9d**, **9e**, **9j** and **9l** antimicrobial agent (II) with dehydrosqualene synthase (PDB: 2ZCS) of *Staphylococcus aureus*.

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Appendix A. Supplementary data

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